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SICKLING DISORDERS

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MEMBRANE-ASSOCIATED SICKLE HEMOGLOBIN: A MAJOR DETERMINANT OF SICKLE ERYTHROCYTE RIGIDITY. E. Evans* and N. Mohandas, Dept. of Pathology, Univ. of British Columbia, Vancouver, Canada, and Dept. of Lab. Medicine, Univ. of California, San Francisco, CA.

In previous micropipet aspiration tests on single red cells, we found that the static rigidity (i.e., membrane shear modulus) of sickle cells increased with increasing cell hemoglobin concentration whereas the rigidity of normal cells was independent of hemoglobin concentration. Another significant feature was that sickle cells exhibited persistent or residual deformation after mechanical extension more frequently, and to a greater extent, than normal cells. To ascertain if differences in hemoglobin association with normal and sickle cell membranes could account for these observations, we measured the extensional rigidity of single red cells which had been prepared according to the following procedure: Normal and sickle cells with defined hemoglobin concentration were selected from discontinuous Stractan density gradients, then lysed in hypotonic medium and reconstituted with the opposite type of hemoglobin solution. The reloading procedure was successful because normal ghosts reloaded with sickle hemoglobin could be sickled by deoxygenation whereas sickle ghosts reloaded with normal hemoglobin could not be sickled. Measurements of static rigidity were higher (13×10^{-3} dyn/cm) for normal red cells ghosts reloaded with sickle hemoglobin than those for either normal red cell ghosts reloaded with normal hemoglobin or native, low- and high-density normal cells ($7\text{-}8 \times 10^{-3}$ dyn/cm). On the other hand, the increased rigidity of high-density, native sickle cells ($16\text{-}25 \times 10^{-3}$ dyn/cm) decreased to near-normal values ($8\text{-}10 \times 10^{-3}$ dyn/cm) after reconstitution with normal hemoglobin. Further, we observed that normal ghosts reconstituted with sickle hemoglobin exhibited persistent (residual) bumps after mechanical extension, but no bumps were formed on normal ghosts reconstituted with normal hemoglobin. Moreover, residual bumps were not produced on low-density sickle cells (which usually exhibited persistent bumps after mechanical deformation) which were reloaded with normal hemoglobin. Since mechanical characteristics peculiar to sickle cells could be induced in normal cells by incorporation of sickle hemoglobin and normal characteristics could be restored to sickle cells by incorporation of normal hemoglobin, we suggest that interaction of sickle hemoglobin with the cell membrane and not intrinsic defects in membrane structure is responsible for sickle cell rigidity.

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SICKLE CELL TRAIT IN ASSOCIATION WITH HB H: A NEW PATIENT FROM THE MEDITERRANEAN REGION. A.E. Felice, A. Kutlar,* M. Kumi,* C. Altay,* A. Gurgey,* and Y. Kilinc.* Comprehensive Sickle Cell Center, Departments of Cell and Molecular Biology and Pediatrics, Medical College of Georgia and Hemoglobin Research Laboratory, Veterans Administration Medical Center, Augusta, GA., and Cukurova University, Adana, and Hacettepe University, Ankara, Turkey.

Only five Hb S heterozygotes with an associated Hb H Disease i.e. the ASH condition or AS: --/-α have been defined in molecular detail. These had ancestries of Saudi-Arabian, Black or mixed Black and Chinese origin. We have studied a sixth patient who was from Turkey. The propositus was detected at birth with Hb Bart's at 27.6%. This declined to 1.6% at nine months, while the proportion of Hb S which was 5.1% at one month increased to 18.0% at one year. At this age, CBC gave Hb = 7.3 g/dl; MCV = 66 fl; and RBC = 4.9×10^{12}/l. DNA of the propositus digested with various enzymes and hybridized with α and ζ probes gave fragments consistent with the common -3.7 Kb α-thal-2 in association with the type of α-thal-1 which is due to a 20.5 Kb deletion with its 5' end 3' to the ζ_2 gene and a 3' end within the α_1 gene. This is the first patient with ASH to have this α globin genotype. The five previously described had combinations of the same type of α-thal-2, but different α-thal-1 genotypes. The proportion of Hb S in these cases fell within the range of 18.0% - 25.0% (20.2% ± 2.6). A notable difference between them is the higher proportion of Hb H (10.0%) reported in patients from Saudi Arabia compared to the others (< 2.0%). A study of additional patients with these combinations is necessary to determine whether the difference is due to different β related haplotypes or different types of α-thalassemia or different analytical methods.

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DEFECTIVE FIBRINOLYSIS IN SICKLE CELL DISEASE. P. Glas-Greenwalt, J. Palascak, R. Gruppo, D. Stroop,* and V. Pollak.* University of Cincinnati Medical Center and Childrens' Hospital Research Foundation, Cincinnati, Ohio.

Vasocclusive crises (VOC) cause significant morbidity and mortality in sickle cell disease (SCD). Although sickling is thought to be the predominant factor in VOC, investigators have examined the possible role of the hemostatic mechanism in the process. The data are, however, inconsistent. We studied, functionally with the fibrin plate method, the fibrinolytic system in 36 adults in the steady state and in 8 children, 7 of whom suffered from painful crises. Values in 240 normal blood donors were: tissue-type plasminogen activator activity (t-PA): 3-25 activator units/ml, corresponding to 0.04 to 0.4 ng/ml; plasminogen activator inhibitor (PA-I): 649-885 inhibitor units/ml; and α_2-antiplasmin (α_2-AP): 718-970 inhibitor units/ml. In patients with sickle cell disease t-PA levels were below the normal range in 24/44. PA-I and α_2-AP levels were elevated in 35/44 and 23/44 respectively. The prevalence was similar in patients in crises and quiescent. Functionally impaired t-PA was associated with either low, normal or high t-PA antigen levels measured immunologically with an ELISA technique (normal range: 3-6 ng/ml). This indicates various degrees of endothelial dysfunction, ranging from impaired synthesis to functionally defective protein to complex formation with PA-I. Fibrin has been implicated in the intravascular sludging of sickle cells. It is suggested that a defective fibrin-clearing system contributes to the syndrome of SCD.

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Natural Killer (NK) Cell Activity in Sickle-Cell Patients. *Gollapudi S., *Ghoneum M.,*Gill G.,*Thadepalli H.,*Hyun M., Chillar R. King/Drew Medical Center and UCLA School of Medicine, Los Angeles, Calif.

The immune status of ten sickle cell patients in steady state was examined. The proliferative response of peripheral blood mononuclear cells (MNC) to OKT3 monoclonal antibody, which closely mimick the activation of T-cell by antigen, and to polyclonal T-cell mitogen Con-A was not altered. However, the B-cell response to anti-antigen receptor antibody (anti-immunoglobulin) was significantly higher than the age matched controls. All patients exhibited a significant higher than the age matched controls. All patients exhibited a significant decrease in their natural killer (NK) cell activity ($P < 0.001$) when compared to healthy controls. This decrease in NK activity is associated with decreased binding of NK cells to target cells. These findings are considered in terms of possible immunoregulatory defects in Sickle Cell patients which leads to increased incidence of infections in these subjects.